

Behavioral and Neurological Toxicity of Polybrominated Biphenyls in Rats and Mice

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Male, albino rats of the F-344/N strain and mice of the B₆C₃F₁ strain were dosed by gavage, 5 days per week for a total of 22 doses with 0.03–30 mg/kg of FireMaster FF-1, 0.168–16.8 mg/kg of 2,4,5,2',4',5'-hexabromobiphenyl (HBB), or corn oil vehicle. A battery of tests was administered at the end of repeated dosing (30 day examination) and 30 days after dosing ceased (60 day test). FF-1 and, to a much lesser extent, HBB decreased body weight and performance on a variety of tests designed to detect neuromuscular dysfunction. Included in these tests were activity in the open field, forelimb grip strength, and muscular reflexes. Visual placement responses were also decreased in some animals, while hypothermia was observed in others. Emotionally, as measured by the number of defecations and urinations in the open field, was not affected by exposure to either compound.

At the 30 day test, mice were less affected by exposure to these polybrominated biphenyls (PBBs) than rats; rats tended to worsen during the 30 days of no dosing, while mice tended to improve. These experiments indicate that oral dosing with levels of PBBs below those required to produce signs of acute toxicity produces behavioral or neurological toxicity when given repeatedly.

Introduction

FireMaster FF-1 or BP-6 is a mixture of polybrominated biphenyls (PBBs) that is used in certain plastic products as a fire retardant. Large numbers of people have been exposed to low amounts of these chemicals following the accidental mixing of the PBBs into livestock feed in Michigan during 1973 and 1974. The long-term health hazards resulting from this incident are currently being assessed (1, 2).

Studies on the acute and subacute toxicity of the PBBs in animals have indicated that they can produce marked morphological and functional changes in at least the liver, thyroid, and testes (3, 4). Other prevalent signs of PBB toxicosis in animals include lacrimation, disturbances in the estrous cycle, lameness, increased frequency of urination, and dermatological abnormalities (1, 5). Clinical observations of both humans and animals have also

suggested that exposure to PBBs may result in behavioral or neurological deficits (2, 5). However, the precise dose and duration of exposure to the PBBs in these reports were indeterminable.

The purpose of the present research was to evaluate some behavioral and neurological effects of known amounts of PBBs given to laboratory rats and mice. In the following experiment, animals were given various doses of FF-1 and 2,4,5,2',4',5'-hexabromobiphenyl (HBB), the main component of the FF-1 mixture. A battery of tests was used to assess behavioral and neurological toxicity at the end of a 30-day dosing regimen, and, as a means of determining the duration of any effects noted or the delayed onset of any signs, 30 days after cessation of dosing.

Methods

Subjects

Male and female rats of the Fisher 344/N strain and mice of the B₆C₃F₁/N strain were obtained at approximately 5–6 weeks of age from the National

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Cancer Institute. The animals were coded for individual identification by means of numbered ear tags. Groups of three to six animals were housed in plastic home cages in a room having a constant light-dark cycle (light, 7 AM to 7 PM), temperature ($21 \pm 2^\circ\text{C}$) and relative humidity ($50 \pm 10\%$). Rat chow (NIH diet #31) and water were freely available throughout the experiment.

Dosing and Procedure

FireMaster FF-1 (FireMaster BP-6 plus calcium trisilicate, Michigan Chemical Co., lot no. 1312 FT) and HBB (synthesized and checked for purity by the Chemistry Branch at NIEHS) were suspended in corn oil and given by gavage, 5 days per week for a total of 22 doses. Rats were randomly assigned to groups receiving 0.03, 0.30, 3.0, or 30 mg/kg-day of FF-1, 0.168, 1.68, or 16.8 mg/kg-day of HBB, or 1 ml/kg-day of corn oil vehicle. Doses were based on body weights taken once weekly. Mice were given 3 or 30 mg/kg-day of FF-1, 16.8 mg/kg-day of HBB, or corn oil vehicle. Six animals of each sex were assigned to each dose group and 12 animals of each sex to the vehicle groups.

Approximately 2 hr after the twentieth and twenty-first doses on days 28 and 29 of the experiment, the subjects were tested for behavioral and neurological dysfunction by a battery of tests (30 day test). After receiving one more dose on the following day, all administration of PBBs was terminated for a period of thirty days. On days 58 and 59 of the study, all surviving animals were re-examined in the battery of tests (60 day test).

Test Battery

Seven measures were taken over a period of 2 days in a fixed order during the 30 and 60 day tests. All subjects in a home cage were examined before proceeding on to the next task in the sequence. Behavioral testing was conducted on a blind basis and each task was administered by the same experimenter during both evaluation periods.

Body Weight. Prior to the study, the animals were ranked according to body weight and randomly assigned to matching groups. Body weights were taken during the week of the 30 and 60 day tests.

Reflexes. The evaluation of reflexes to externally applied stimuli was the first behavioral test that was administered. Due to the time required to complete this portion of the battery, only those rats and mice receiving 3 and 30 mg/kg-day of FF-1, 16.8 mg/kg-day of HBB or corn oil vehicle were

examined.

At the start of the test, each subject was lifted from the home cage by the experimenter and tested for the pinna (ear twitch) and eye blink reflexes as described by Irwin (6). The subjects were then held by the nape of the neck, and the lateral surfaces of the middle toe of each hind leg were compressed with a pair of forceps (ipsilateral flexor reflex). Next, the subjects were grasped by the tail and, at a point approximately three-fourths of the way up the tail from the tip, pressure was applied with a pair of forceps. In all cases, an experienced rater determined whether or not the flexes were normal or depressed. As described by Irwin (6), a cage of animals known to be untreated was used as a reference group to aid in the rating of depressed responses.

Visual Placing. After being examined for reflexes, the subjects were picked up by their tail and lowered nose-first from a height of about 15 cm toward a wire grasping ring (see grip strength test). The animal was rated as having made a visual placing response if it extended its head and forelimbs toward the grasping wire before the nose or whiskers contacted the ring.

Grip Strength. Muscular strength in the forelimbs was measured using a recording grip meter described in detail elsewhere (7). Briefly, the animals were held by the tail and allowed to grasp a wire ring 45 mm in diameter connected to a push-pull strain gauge by means of a connector rod supplied by the maker (J. Chatillon and Sons, Inc., Kew Gardens, N. Y.). After grasping the wire, the hindquarters of the animal were rotated into a horizontal attitude by the experimenter. The subject was then pulled until it released the wire. The force in grams measured by the strain gauge was then recorded. The average of three trials in which the subject pulled with a constant force using both forelimbs was taken as the measure of grip strength.

Rectal Temperature. The animals were restrained for a 1-2 min period and rectal temperature was measured by a Yellow Springs tele-thermometer (Model 44TA).

Open Field Activity. The motor activity of rats was measured in a Plexiglas open field maze (3 ft \times 3 ft \times 1 ft deep) marked off into 36 equal segments with black paint. At the start of the test, the subjects were placed in one of the four corners with its head facing the wall. The number of lines crossed during a 3 min period was counted. Mice were similarly tested in an open field maze having smaller dimensions (18 in. \times 18 in. \times 1 ft deep).

Emotionality. The number of defecations and urinations occurring during the 3 min observation period in the open field maze was determined as a measure of emotionality (8).

Statistical Analyses

A mixed model analysis of variance (ANOVA) for repeated measures (9) was used where appropriate to evaluate overall effects of treatment (exposure to PBBs), sex, day of testing, and interactions between fixed variables. If a significant treatment effect was observed, difference between means of treated subjects and controls were tested for statistical significance by means of Fisher's least significant difference tests (9). In the case of the visual placement response and motor reflexes, which are quantal or all or none measures, differences between control and treated groups were evaluated for statistical significance by Fisher's exact probability tests (10). The accepted level of significance was $p < 0.05$, using two-tailed tests.

Whenever the PBBs produced significant effects in rats, they were most consistently observed at 3 and 30 mg/kg-day for FF-1 and 16.8 mg/kg-day for HBB. Any effects that were seen at lower doses were always in the same direction of those at higher doses. Thus, for the sake of brevity and for a more concise comparison of rats with mice, subsequent discussion of differences between individual treatment groups and controls will be confined to the two higher doses of FF-1 and the high dose of HBB.

Results

Body Weight

Inspection of the body weights recorded during the 30 and 60 day tests and subsequent ANOVA of these data indicated that treatment with PBBs decreased body weights significantly ($F = 15.0$; $df = 7/92$; $p < 0.0001$). As expected, the male rats tended to weigh more than the female rats ($F = 1101.0$; $df = 1/92$; $p < 0.0001$), particularly at the 60 day test ($F = 1187.0$; $df = 1/92$; $p < 0.0001$). However, male rats were affected more by exposure to PBBs than the females ($F = 3.6$; $df = 7/92$; $p < 0.001$). The weight decreasing effect of the PBBs was also greater at the 60 day test than at the 30 day test ($F = 4.9$; $df = 7/92$; $p < 0.0001$).

Pairwise comparison of treated and control groups indicated that 30 mg/kg-day of FF-1 significantly decreased the body weight of male and female rats at the 30 and 60 day tests (Fig. 1). The 3 mg/kg-day dose of FF-1 and 16.8 mg/kg-day of HBB had no significant effects on body weights.

The body weight of mice was also affected significantly by exposure to PBBs ($F = 4.07$; $df = 3/48$; $p < 0.015$). As in the case of the rats, male mice weighed more than females ($F = 164.4$; $df = 1/48$; $p < 0.0001$) and the difference was

greater at 60 days than at 30 days ($F = 5.85$; $df = 1/48$; $p < 0.02$). Male mice were also affected by PBBs more than females ($F = 3.56$; $df = 3/48$; $p < 0.025$). Body weights of mice of both sexes were higher at the 60 day test than at the 30 day test ($F = 241.5$; $df = 1/48$; $p < 0.0001$) and the treatment effect was greater at 60 days than at 30 days ($F = 3.06$; $df = 3/48$; $p < 0.04$). However, the sex by day by treatment interaction was not significant ($F = 0.97$).

Pairwise analysis of control and treated groups of Dunnett's *t*-test indicated that female mice were not affected by the higher concentration of FF-1 or by HBB. Male mice receiving 30 mg/kg-day of FF-1, however, weighed significantly less than controls at the 30 day test, but not at the 60 day test. HBB had no significant effect on the body weight of male mice at the 30 day test, while males given HBB were significantly heavier than controls at the 60 day test.

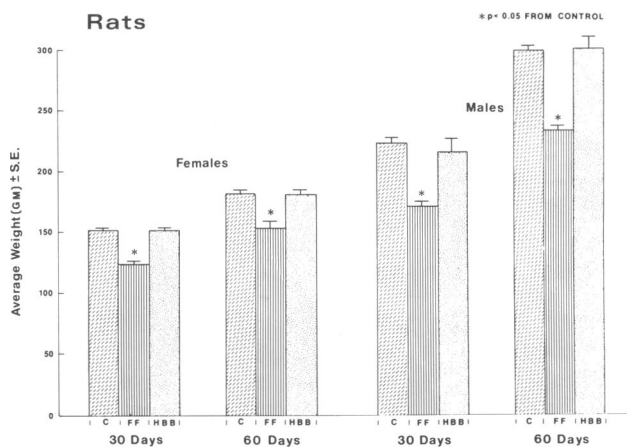


FIGURE 1. Effects of FF-1 (30 mg/kg-day), HBB (16.8 mg/kg-day) or corn oil vehicle on the body weight of female and male rats at the 30 and 60 day tests. There were 12 animals in each control group and 6 animals in each treatment group. The asterisk indicates a statistically reliable difference between control and treated group (Fisher's least significant difference, $p < 0.05$, two-tailed).

Reflexes

When the number of rats rated as having one or more depressed reflexes was compared to the number having all seven reflexes rated as being normal, the PBBs had no effect on reflexes at the 30 day test (Fig. 2). However, at the 60 day test, both 30 mg/kg-day of FF-1 and 16.8 mg/kg-day of HBB produced a significant decrease in the number of male and female rats having reflexes rated as normal. The lower dose of FF-1 (3 mg/kg-day) was without noticeable effect.

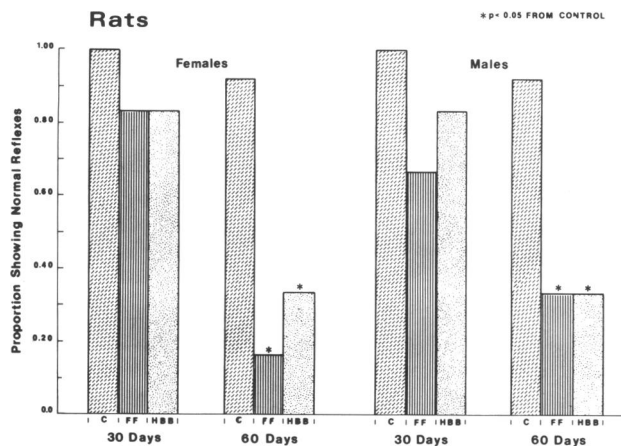


FIGURE 2. Effects of FF-1 (30 mg/kg-day), HBB (16.8 mg/kg-day), and corn oil vehicle on the reflex responses of female and male rats. There were 6 animals in each treatment group and 12 in each control group. The asterisk denotes a statistically reliable difference between the PBB and vehicle-exposed groups (Fisher's exact probability test, $p < 0.05$, two-tailed).

The effect of PBBs on the reflexes of mice were less pronounced than those observed in rats. The only significant decrease in the number of normal reflexes that was observed in mice was that at the 30 day test in female mice given 30 mg/kg-day of FF-1.

Visual Placement

Repeated dosing with either FF-1 or HBB had no significant effect on the visual placement response of female rats or mice. Male rats exposed to 30 mg/kg-day of FF-1 or 16.8 mg/kg-day showed significantly fewer visual placing responses at the 60 day, but not the 30 day test (Fig. 3). Male mice were likewise affected by FF-1 and HBB, except the effect was significant at 30 days, but not 60 days.

Grip Strength

Exposure to PBBs decreased grip meter scores of rats significantly ($F = 12.0$; $df = 7/92$; $p < 0.0001$). Male rats had significantly higher grip scores than females ($F = 47.0$; $df = 1/92$; $p < 0.0001$), and the difference did not vary according to the day of test ($F = 1.8$; $df = 1/92$). Male rats were not affected more than females by exposure to PBBs ($F = 1.2$; $df = 7/92$). Rats tended to get stronger between the 30 and 60 day tests ($F = 273.0$; $df = 1/92$; $p < 0.0001$) and the effect of PBBs at the 60 day test was greater than at the 30 day test ($F = 3.4$; $df = 7/92$; $p < 0.003$). The effect of the PBBs was not dependent upon the combined effects of sex and day of testing ($F = 1.1$; $df = 7/92$).

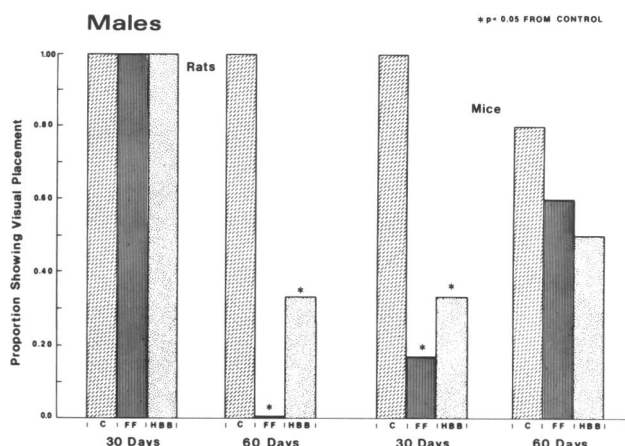


FIGURE 3. Effects of FF-1 (30 mg/kg-day), HBB (16.8 mg/kg-day), and corn oil vehicle on the visual placement response of male rats and mice at the 30 and 60 day tests. There were 6 animals in each treatment group and 12 in each control group. The asterisk denotes a statistically reliable difference between the PBB and vehicle-exposed groups (Fisher's exact probability test, $p < 0.05$, two-tailed).

Pairwise comparisons of the grip meter scores of controls and PBB-exposed rats indicated that 30 mg/kg-day of FF-1 decreased the grip strength of both males and females at the 30 and 60 day tests (Fig. 4). Exposure to 16.8 mg/kg-day of HBB produced a significant decrease in the grip strength of male rats at the 30 and 60 day tests, while female rats were not affected significantly at either test period.

The grip strength of mice was not significantly affected by exposure to PBBs ($F = 2.5$; $df = 3/48$). Male mice were significantly stronger than females ($F = 9.5$; $df = 1/48$; $p < 0.003$) and grip strength scores were higher at 60 days than at 30 days ($F = 24.4$; $df = 1/48$; $p < 0.0001$).

Rectal Temperature

Treatment with PBBs had no significant effect on the rectal temperature of rats ($F = 1.2$; $df = 7/92$). The temperature of the female rats was significantly higher than the males ($F = 57.3$; $df = 1/92$; $p < 0.0001$), while temperatures of the females on the 60-day test were significantly lower than those observed during the 30 day test ($F = 33.5$; $df = 1/92$; $p < 0.0001$).

Unlike the rats, the PBBs significantly affected the rectal temperature of mice ($F = 9.8$; $df = 3/48$; $p < 0.0001$). Temperatures taken during the 60 day test were lower than those on the 30 day test ($F = 5.7$; $df = 1/48$; $p < 0.02$). There was no significant difference in temperature between males and females ($F = 2.0$; $df = 1/48$). The sex and treatment

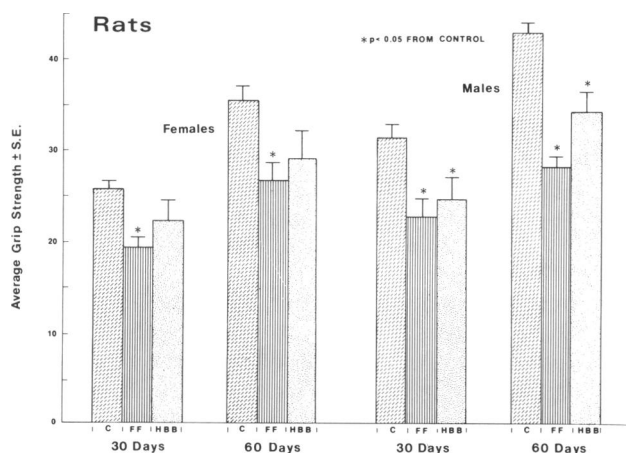


FIGURE 4. Effects of FF-1 (30 mg/kg-day), HBB (16.8 mg/kg-day), and corn oil vehicle on the grip meter scores of female and male rats at the 30 and 60 days of testing. There were 12 animals in each control group and 6 in each treatment group. The asterisk indicates a statistically reliable difference between control and PBB-exposed animals (Fisher's least significant difference, $p < 0.05$, two-tailed).

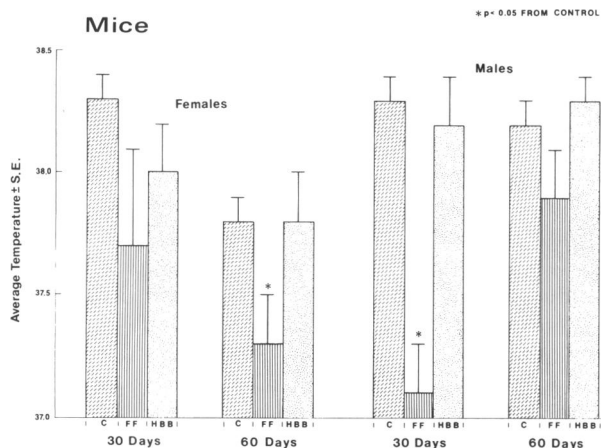


FIGURE 5. Effects of FF-1 (30 mg/kg-day), HBB (16.8 mg/kg-day), and corn oil vehicle on the rectal temperature of male and female mice at the 30 and 60 day tests. There were 12 animals in each control group and 6 in each treatment group. The asterisk indicates a statistically reliable difference between control and PBB exposed animals (Fisher's least significant difference, $p < 0.05$, two-tailed).

($F = 0.7$) and the sex and day and treatment ($F = 1.5$; $df = 3/48$) interactions were not significant, but the sex and day ($F = 9.0$; $df = 1/48$; $p < 0.004$) and treatment and day ($F = 2.8$; $df = 3/48$; $p < 0.05$) interactions were significant.

Pairwise analyses of group and treatment means showed that 30 mg/kg-day of FF-1 decreased body temperature of male mice at the 30 day test, while female mice receiving the high dose of FF-1 were hypothermic at the 60 day test (Fig. 5). HBB had no effect on the rectal temperature of mice.

Activity in the Open Field

The number of crossings made by rats during a 3 min observation period in the open field was decreased by PBB treatment ($F = 3.1$; $df = 7/92$; $p < 0.005$). Female rats were more active than males ($F = 26.4$; $df = 1/92$; $p < 0.0001$) and were affected by PBB treatment more than males ($F = 2.4$; $df = 7/92$; $p < 0.025$). Activity at 60 days was lower than at 30 days ($F = 30.3$; $df = 1/92$; $p < 0.0001$) and the effect was observed equally in both males and females. That is, there was no significant sex by day interaction ($F = 1.4$; $df = 1/92$). The effect of PBB on activity also did not vary significantly according to test day ($F = 1.6$; $df = 7/92$) nor did it vary according to the sex of the subject and the day of testing ($F = 1.4$; $df = 7/92$).

Female rats given 30 mg/kg-day of FF-1 and 16.8 mg/kg-day of HBB had reduced activity in the open field at the 30 and 60 day tests (Fig. 6). The open field activity of male rats was affected significantly only at 30 days in subjects receiving 16.8 mg/kg of HBB.

Although mice treated with PBBs tended to have decreased activity scores, ANOVA indicated no significant treatment effect ($F = 1.9$; $df = 3/48$). There was, however, an indication that females were more active than males ($F = 6.7$; $df = 1/48$; $p < 0.02$) and that there was a downward shift in activity from day 30 to day 60 ($F = 32.7$; $df = 1/48$; $p < 0.0001$). Thus, in terms of the motor-activity decreasing effects of PBBs, the mice were clearly less affected than rats.

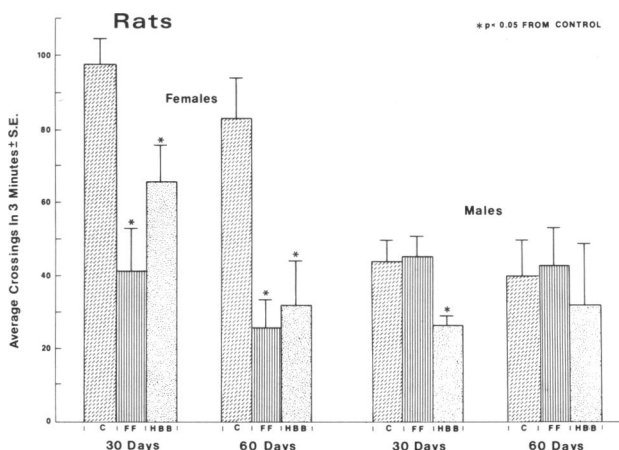


FIGURE 6. Effects of FF-1 (30 mg/kg-day), HBB (16.8 mg/kg-day), and corn oil vehicle on the motor activity of rats in the open field. There were 12 animals in each control group and 6 in each treatment group. The asterisk indicates a statistically reliable difference between control and PBB exposed animals (Fisher's least significant difference, $p < 0.05$, two-tailed).

Emotionality in the Open Field

Treatment with PBBs had no significant effect on the number of defecations ($F = 1.47$; $df = 7/92$) or urinations ($F = 1.41$; $df = 7/92$) by rats during a 3 min observation period in the open field. However, male rats defecated ($F = 26.8$; $df = 1/92$; $p < 0.0001$) and urinated ($F = 20.9$; $df = 1/92$; $p < 0.0001$) more than female rats, while there were more defecations ($F = 7.10$; $df = 1/92$; $p < 0.0001$) on day 30 than on day 60. The number of urinations did not differ according to day of testing ($F = 0.25$).

The number of defecations ($F = 0.86$) and urinations in mice ($F = 1.10$; $df = 3/48$; $p < 0.05$) were also not affected by exposure to FF-1 and HBB. Concurrent effects of sex or day of treatment on defecations and urinations of mice were not observed.

These data indicate that emotionality, as measured by the number of defecations and urinations occurring in the open field, was not affected by treatment with FF-1 or HBB in either rats or mice.

Discussion

The results of the present investigation indicate that PBBs given orally to mice and rats over a period of 30 days result in decreased body weight, depressed motor reflexes, impaired forelimb grip strength, and decreased motor activity. Such effects were noted in rats receiving a total dose of 100-150 mg of FireMaster FF-1 during 30 days of dosing. Mice, which were approximately one-tenth as heavy as the rats and received a total dose of only 10-15 mg, were clearly less affected by the PBBs.

In the case of the rats, the signs that were present at the 30 day test were still evident 30 days after cessation of dosing (60 day test). The mice, however, did not tend to change following the termination of PBB exposure. These observations, taken with the fact that the mice were generally less impaired than the rats prior to cessation of dosing, indicate that the duration of the adverse effects may be in part dependent upon the severity of the symptomatology at the end of the exposure period. The data also indicate that repeated exposure to concentrations of PBBs below those required to produce signs of acute toxicosis may result in signs having an indeterminate duration. In order to assess the reversibility of effects such as those observed in the present experiment, studies are needed which evaluate behavior at longer times after cessation of dosing.

It was noteworthy that administration of 2-, 4,5,2',4',5'-hexabromobiphenyl (HBB) in concen-

trations equal by weight to the HBB in the FireMaster FF-1 mixture (16.8 mg/kg-day as compared to 30 mg/kg-day, respectively) had few effects on the behavior of rats and mice. These data indicate that the other components contained in the FF-1 mixture, i.e., other hexabrominated isomers, penta- and heptabromobiphenyls, contribute to the toxic effects of FF-1. Additional studies are, therefore, needed to establish the toxicity of these agents.

In the present experiment, exposure to PBBs resulted in decreases in body weight, which is consistent with the findings of other investigators. Jackson and Halbert (5), for example, observed decreases in the body weight of dairy cattle that had ingested feed contaminated with FF-1. The addition of FF-1 to the feed of chickens (11) and Japanese quail (12) has been reported to produce decreases in body weight and signs of inanition. The average weight gain of rats has also been reported to decrease following dietary exposure to 100-500 ppm of PBBs for 30 days (3). It is interesting to note, however, that humans exposed to PBBs did not report anorexia and weight loss (2). It seems likely, therefore, that loss of body weight or failure to gain weight is a manifestation of exposure to relatively high doses of PBBs.

The decreases in motor function we observed in the grip strength test, the reflex examination and in open field activity are in accord with the reports of other investigators (5). Meester and McCoy (2) also conclude that complaints involving the skeletoneuromuscular system represent some of the more consistent symptoms reported by humans exposed chronically to PBBs. Changes in skeletomuscular functions may, therefore, represent a symptom indicative of exposure to low to moderate amounts of PBBs.

The mechanism by which the PBBs produce decreases in body weight and alterations in neuromuscular function is presently unclear. However, PBBs have been shown to induce microsomal hepatic enzymes and produce hepatohistopathology at concentrations similar to those used in the present study (3). Exposure to PBBs has also been found to suppress immune responses in rats (13), and Ringer and Polin (4) have suggested that animals exposed to PBBs may suffer from hypothyroidism. Thus, the deleterious effects of PBB administration observed in the present work may in part be due to adverse effects on the general health of the animals. The inanition observed in chickens and Japanese quail fed PBBs tends to support this conclusion (11, 12).

That the visual placement response was affected in some animals exposed to PBBs suggests a deficit in sensory-perceptual functioning. However, there

is a substantial motor component in the placement response. Thus, PBB-induced dysfunction of the skeletoneuromuscular system could have contributed to the disturbance of this response. Further experiments using more sensitive techniques are needed to clarify this issue.

Exposure to PBBs resulted in decreased rectal temperatures of mice, but rats were not affected. Since the rectal temperatures of dairy cattle poisoned with PBBs have been reported to be unaffected (5), the relevance of our finding with mice remains to be determined.

Our results concerning defecations and urinations in the open field suggest that PBB exposure did not influence emotionality. Gross observations of laboratory animals receiving PBBs have not indicated changes in such behavioral variables (3, 4). However, future experiments using more precise measures of emotionality and irritability would be of interest.

In summary, oral administration of FF-1 over a period of 30 days produced decreases in body weight and decreases in the motor functioning of rats, and, to a lesser extent, mice. In rats markedly affected by exposure to the PBBs, symptoms of toxicity were still prevalent 30 days after cessation of dosing. The mechanism by which exposure to PBBs produces the behavioral effects observed in the present study remains to be elucidated and serves as a stimulus for future research.

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